THESIS FOR THE EXAMINATION FOR THE DEGREE OF M.D., EDIN.

ENTITLED

"THE FOLLICLE CELLS OF THE MAMMALIAN OVARY, WITH SPECIAL REFERENCE TO THE SUPPOSED RELATION BETWEEN THE SEXUAL GLANDS AND THE SO-CALLED MALIGNANT NEOPLASMS"

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Declaration

I hereby declare that this thesis has been composed by myself unaided, and that the investigations upon which it is based have been carried out by myself. I also declare that this thesis is printed solely for the purposes of the examination for the degree of Doctor of Medicine, that it has not been published and that it is not intended to publish it.

(Signed) V. Chastel de Boinville.

April 5. 06.

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INDEX TO THIS THESIS

"THE FOLLICLE CELLS OF THE MAMMALIAN OVARY"

BY

V. CHASTEL DE BOINVILLE.

The object of this paper is, first, to state as briefly as possible the results, in so far as they have been attained, of certain investigations carried out by the author into the nature of the follicle cells of the mammalian ovary, and, secondly, to endeavour to establish more clearly those obscure connections which have been long suspected by Beatson[1] and others to exist between the growth of malignant tumours and the development of the sexual glands. The investigations referred to were undertaken by the writer in the laboratories of the Liverpool Cancer Research during the years 1904 and 1905. In addition to the cytological work, which will be hereunder described in detail, a passing reference will be made to some experiments, connected with the transplantation of ovaries, which were carried out during the same period, with a view to establishing the functional as well as the structural nature of these cells.

In the present paper the nature of the follicle cells is not discussed from the standpoint of an embryologist *primâ facie*, though a few remarks will be made in this direction with a view to throwing light on the nature of the follicle cells in connection with their relation, if any, to the oöcytes around which they develop. Nor is it intended to offer more than a few suggestions as to their possible significance regarding those obscure functions of the ovary, connected with sexual characteristics, which are generally attributed to internal secretions on the part of this gland. As regards cancer a few remarks will be made in relation to the significance of the investigation to be described in so far as it touches upon certain already current theories in connection with cancer genesis, while the theory which attributes the origin of cancer to the germ cell, will, in a general way, be accepted throughout as the fundamental hypothesis of this essay.

The term "follicle cells" is here intended to imply only those cells which go to form the *zona glomerulosa*, and the *discus proligerus* of the Graäfian follicle. The term is not intended to include either the ova or those cells of the interstitium which enter into the formation of the investing layer.

The ovaries examined have been chiefly those of the rabbit, but sufficient attention has been paid to the ovaries of the bitch, cat, sheep and sow, to shew that the follicle cells in all these animals are of the same essential nature, and it is, therefore, reasonable to regard what is to be described as typical of mammals or, perhaps, of vertebrates in general.

Before going any further it would be well to call attention to the fact that there has long been some doubt as to the true nature of the follicle cells. The question has for a long time past resolved itself into two opposite views, the one adopted by Pflüger, that these cells are germinal in character, the other by Waldeyer, that they are true epithelial cells of a somatic nature. Cytologists and embryologists have been divided in opinion. A full discussion on this question will be found in a recent work of Korschelt and Heider.[2] The discovery of the existence of germ cells in various parts of the body, in the embryos of *Raja batis* and other fishes, we owe to Beard,[3] and much of what it may be possible to discuss in this paper depends upon this investigation of his, from which he has drawn his own conclusions in certain of his more recent papers.[4] There has, however, up to the present, been no positive evidence of a structural nature by which the true character of the follicle cells could be identified. Their germinal character is suggested by their general primitive appearance and by their intimate association with the oöcytes which they surround. Retzius[5] and others have endeavoured to demonstrate the existence of intercellular bridges between the oöcyte and the follicle cells, while their common origin from cells included in the "germinal epithelium" of the ovary establishes a further connection between these two elements. Lastly, the much larger number of germ cells to be found in the testis suggests that there may be more germ cells in the ovary than are accounted for by the oögonia and their offspring. It is true that some writers have attempted to explain the larger number of sexual cells that are found in the testicle as compared with that of the ovary of the same species, by the theory of what is known as the physiological division of labour, the large ova containing a greater amount of protoplasm and yelk, the spermatozoa being very small and active; also by the fact that four spermatozoa correspond to one ovum and three polar bodies, the latter being apparently discarded. But the physiological division of labour only partly accounts for the unequal distribution of germinal tissue in the sexual glands of the two sexes, so that this again would point to their being other germ cells in the ovary, over and above those that have already been identified.

The sudden leap from a long period of inactivity into one of active proliferation which forms one of the characteristics of follicle growth, though it is not by any means a phenomenon peculiar to germ cells, is, however, one among other pieces of evidence, which points to the germinal character of this tissue.

The identification of a germ cell, in the higher animals at any rate, is no easy task, unless there can be found within it some characteristic peculiar to this type of cell, or else some character which, while it is known to be peculiar to germ cells in one or other stage of their existence, is unknown in the history of somatic cells.

Heterotype mitoses, by which is here meant a nuclear division carried on with a reduced number of chromosomes, is never found in normal somatic tissue, while it is always found in germ cells during the terminal divisions which give rise to the sexual elements, and also in many cells belonging to the infiltrating neoplasms which are here regarded as being partly or entirely of a germinal nature.

While the majority of germ cells, then, exhibit during their divisions the usual somatic number of chromosomes characteristic of the species in which they grow, the presence of heterotype mitoses occurring among any cells is at once highly suggestive of their germ cell character.

After a careful examination of many hundreds of nuclei belonging to the follicle cells of the mammalian ovary, the writer has been led to believe that the type of mitotic division occurring in these cells is not infrequently heterotype; that is to say, that these cells partake of the nature of germ cells as regards, at any rate, their method of dividing, and that the mitotic figures are not infrequently characterised by exhibiting half, or about half, the usual number of chromosomes.

These chromosomes are of a coarse nature as compared with those that may be seen in the cells of the body generally (Somatic cells), while they resemble closely the heterotype cells of a cancer or sarcoma as described by Farmer, Moore and Walker;[6] the general character and method of division being similar to what obtains in the parent cell of the ovum or spermatozoon.

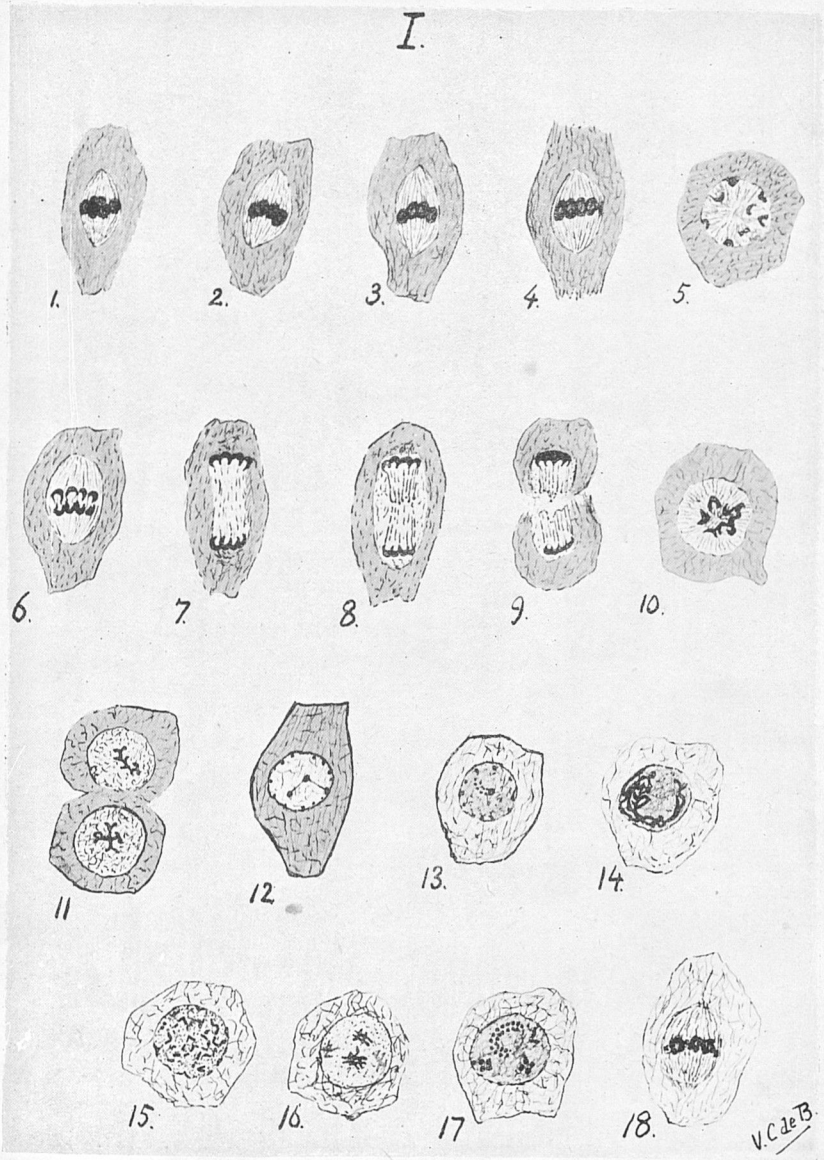
It may be convenient here to note the method of technique employed in preparing the tissue for microscopical examination.

The ovaries were transferred, immediately after killing the animal, to the fixing reagent. Various fixing reagents were used, the best results being obtained with Hermann's platino-osmic-acetic mixture.[7] In one case the fixing was carried out *intra vitam* according to Mann's[8] direction with a mixture of picric acid, corrosive sublimate, formol and water. The stain employed was chiefly iron haematoxylin, the process being that recommended by Heidenhain.[9]

Owing to the smallness of the follicle cells and to the minute size of the chromosomes decolourisation was in some cases carried out almost to vanishing point, the position of the mitotic figures being previously marked. By this means a more accurate opinion could be formed as to their delineation, shape, and disposition.

The coarse nature of the chromosomes in certain of these follicle cells has already been referred to. As seen *en masse* in metaphase, anaphase or telophase, this character becomes the more obvious when compared with similar phases as revealed in a Somatic cell (see Plates I. and II.). The polar views in the heterotype cell shew the distribution of the individual chromosomes more clearly, and their reduced number becomes more apparent. By counting the chromosomes of such cells in serial sections, this number, in the case of the rabbit, would appear to be about eight, or rather more. That is to say, eight chromosomes are distributed to each pole respectively. The exact number, however, was very difficult to determine, but the evidence is sufficient to shew that the number approximately corresponds to the reduced or heterotype number and not to the full somatic number as seen in somatic cells, or in cells of the body generally. The individual chromosomes are comparatively large as compared with the somatic type, their apices are rounded, not sharp and pointed. When arranged at the equator in metaphase they appear as small spherical or disc-shaped bodies, sometimes, in more fortunate specimens, they suggest ring-shaped bodies. In anaphase the chromosomes present the appearance of horseshoes, though they are often irregular in that one limb may be longer than the other.

The obscure phases of mitosis which are generally combined under the convenient term of "prophase," are always difficult to determine in all tissues, and in the case of the follicle cells the task has been an exceedingly difficult one. The following account is, therefore, offered with all reserve. As far as the writer's observations go the process would sometimes seem to resemble that which leads to "tetrad" formation in a parent sexual cell. A coarse spireme thread is formed which splits throughout its length after the usual manner, and the double coil of chromatin subsequently arranges itself in an arc nearly surrounding the central area of the nucleus. This coil is subsequently broken up into a number of short rods which are diffused broadcast over the nucleus. These rods later on accumulate towards the centre of the cell, becoming shorter, thicker and fewer in number, and they then appear to arrange themselves into compound chains composed of a series of minute chromatin bodies, while in the same nucleus where such chains may be seen one or more groups may appear consisting of four or sometimes five small dense masses. The process would thus seem to bear some slight resemblance to the Arthropod type. These masses would then appear to again accumulate each into one dense mass, and to take up a position at the equator, forming a circumferential series of rounded



Cells of the Maturing Follicle from the Ovaries of the adult Rabbit, shewing some of the successive phases of Heteromitosis.

EXPLANATION OF FIGURES.

1—5. Metaphase.
1, 2, 3. and 4. Ring shaped chromosomes.
5. Metaphase in polar view.

6—10. Anaphase and Telophase.
9. Daughter nuclei "shunting" from one another.

11. Daughter Cells immediately after division.

12—13. Resting Cells.

14—18. Prophase.
14. Contracted Spireme.
15. Formation of short rods.
17 and 18. Suggesting "tetrad" formation.

Drawn from Cells stained by Heidenhain's method.

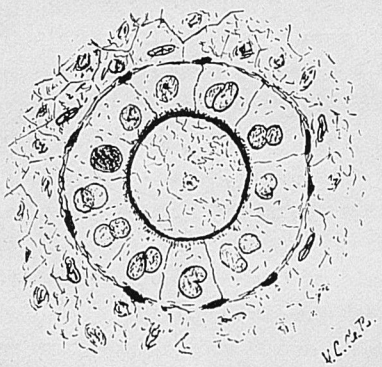
chromosomes partly overlapping one another. So firmly constituted are these chromosomes, that in a cell which has been torn or injured by faulty preparation of the section, they still retain their rounded form and maintain their relation to one another even when the rest of the nucleus is distorted from its usual shape.

As the chromosomes thus formed arrange themselves at the equator in metaphase, they would seem to become rarified in the centre sometimes to such an extent as to give the appearance of rings. The later phases then succeed one another as already described.

The arrangement of the chromosomes in metaphase is nearly always regular, and the appearance of one or more chromosomes having dropped out, such as is so often seen in malignant tumour cells, is seldom if ever to be seen in follicle cells. In anaphase the numerical division of the chromosomes is also regular, in that the same number of bodies are drawn to either pole; otherwise the whole process resembles closely that which is characteristic of the heterotype cells of malignant tumours, especially those of the sarcomata, and forms a link of a striking character between the resemblances exhibited between the cells of these growths and those of the true reproductive or gametogenic cells, while the whole history of the Graäfian follicle, as will be hereafter shewn, forms a parallel to that of the early malignant neoplasm. After the *liquor folliculi* is expelled the follicle cells, as is well known, cease to divide, and under obscure influences, which are perhaps connected with the pelvic congestion which occurs at this period, they go to make up the *lutein cells* of the corpus luteum or yellow body. Many, however, are discarded from the ovary, with the *liquor folliculi*.

As further evidence of the germ cell nature of this tissue it may be noticed that in certain very young follicles—those that consist of a single layer of follicle cells surrounding an ovum, and which are apparently at a stage immediately preliminary to assuming active growth—the nuclei are sometimes duplicated (Plate III.). That is to say, that within the confines of one cell there may be two nuclei lying adjacent to one another. Sometimes they are apparently in apposition and sometimes they are actually fused into one irregular nucleus. These appearances are largely due to the fact that the arrangement is such that one section passes through the individual duplicates in different planes within the same follicle. This irregularity, as will be shewn later, subsequently gives way to a more regular disposition, which is again upset, when, in the course of maturation the ovum leaves the centre of the follicle and takes up an eccentric position. The duplicated nuclei are frequently bent and irregular in shape, their nuclear membrane is well defined, and the disposition of chromatin, and its affinity for basic dyes appear to differ in some slight degree from that of the later follicle cells. This last remark applies also to the very earliest follicle cells which exist definitely as such. These early follicle cells are smaller than their offspring and their nuclei are proportionately more minute.

III.



Young Graäfian Follicle of Rabbit's Ovary.

Shewing duplication of nuclei in different planes, and dividing or resolving by a direct process. Above and to the left, a nucleus is in the prophase of mitotic division.

It is not intended in this paper to discuss the general significance of the last-mentioned observation. Suffice it to say that duplication of nuclei is a characteristic of germ cells, and that it is generally regarded as a resolution of a germ cell into its two constituent halves. Its existence in the *zona glomerulosa* of the young follicle, taken together with the evidence of the heteromitosis which this tissue exhibits, may be regarded as proof of the non-somatic nature of the follicle, while the separation of the duplicated nuclei, where such occurs, may be regarded as a precursory phenomenon to the reduction of chromosomes.

The significance of these observations, in so far as they go, may be calculated to form a new factor in more than one branch of scientific research, among which may be mentioned the problems of oögenesis, the obscure functions of the ovary connected with the sexual characteristics of the female, the nature of the corpus luetum, which is largely constituted of the substance of these follicle cells, the abnormal heteromitosis of malignant tumours, and with the character of heteromitoses in general. If we could arrive at a clear comprehension of the meaning of the last-mentioned process we should have gone far to elucidate many problems connected with the rest. Great and important questions again arise:—What does heteromitosis signify? What determines the reduction of chromosomes? How and when do cells whose chromosomes are thus reduced cease to divide? Why do they in the case of the gameocytes remain quiescent until by fertilisation or conjugation with another cell their chromosomes are replenished? Why are they, in the case of cancer, associated directly or indirectly with indefinite growth, and in the case of the follicle, what is the influence that brings the growth of such a tissue to an abrupt end, after the expulsion of the ovum from the follicle?

There is some evidence that germ cells are structures which, under favourable circumstances are naturally endowed with the essence of everlasting life, the individuals in which they live being merely successive generations of temporary hosts. If this theory (which we associate with the name of Beard[10]) be accepted, the above question may be put more shortly. What checks the division or growth of the germ cell temporarily, as in the case of the unfertilised ovum and in the primary follicle cell? What checks it permanently, as in the case of the ripened follicle? If cancer cells are to be regarded as germ cells, then we may further ask can this checking influence be brought to bear upon cancer?

It is not intended to discuss the many and varied hypotheses which from the time of Roux[11] onwards have been elaborated with a view to answering some of these questions, by investigating the nature of the polar bodies, the methods of chromosome reduction, and of "tetrad" and "dyad" formation, or to do more than submit that a new factor arises among the many that must be associated

with these intricate problems. Furthermore, it is admitted that these phenomena are after all but outward manifestations of a still more intimate process associated with the "memories" that are retained from earlier generations of cells.

A question of more immediate interest is that of the relation of the follicle cells to the oöcyte.

Having regard to the nature of the follicle cells as described above, and remembering the intimate anatomical relation to the ovum around which they cluster, it is probable that these cells have sprung from a comparatively recent ancestor common to them and to the ovum. It is remarkable that their period of existence in the individual who forms their host is practically synchronous with that of the ovum. As the lutein cells of the corpus luteum they still maintain their vitality while the ovum is alive in utero; and if the ovum is rejuvenated by fertilisation it is not until after the embryo is completed that the yellow substance is reduced to a scar.

Further, the suggested relation of follicle cells to ovum in the mammal finds a parallel among certain of the lower orders. For example, in Dytiscus we find according to Giardina[12] that the oögonium divides four times successively giving rise to one oöcyte and fifteen follicle cells. These fifteen follicle cells then divide twice, so that eventually there are sixty follicle cells to one oöcyte. Julin[13] describes a still more interesting development in Styelopsis Grossularia (see fig. I.). In this species the primordial germ cell divides many times to form a large number of oögonia and primary follicle cells. The family tree is such that as each oöcyte is formed there is associated with it three primary follicle cells, one descended from the same parent and two from the same great-grandparent. From these three primary follicle cells are derived a group of follicle cells that are associated with the oöcyte. The oöcyte like that of a mammal divides twice giving rise to one ovum and three polar bodies. In any one group the follicle cells tend to be more closely related to the ovum of that group than to the ovum of any other group. A glance at the figure (1) would perhaps suggest that the distribution of chromatin which determines the different characters of the oöcyte on the one hand and of the follicle cells on the other, occurs *at the latest* by the division that takes place in the parent of the primary oöcyte.

The observations described above would suggest that much the same arrangement may prove to be the case in the mammal (fig. 2). It would seem as if there were a struggle for supremacy among the earlier oögonia of the embryonic ovary, a struggle by successive cell divisions for the retention of certain coveted particles or characters. The successful cell would thus be destined to form the oöcyte with a fair promise of eternal life by the perpetuation

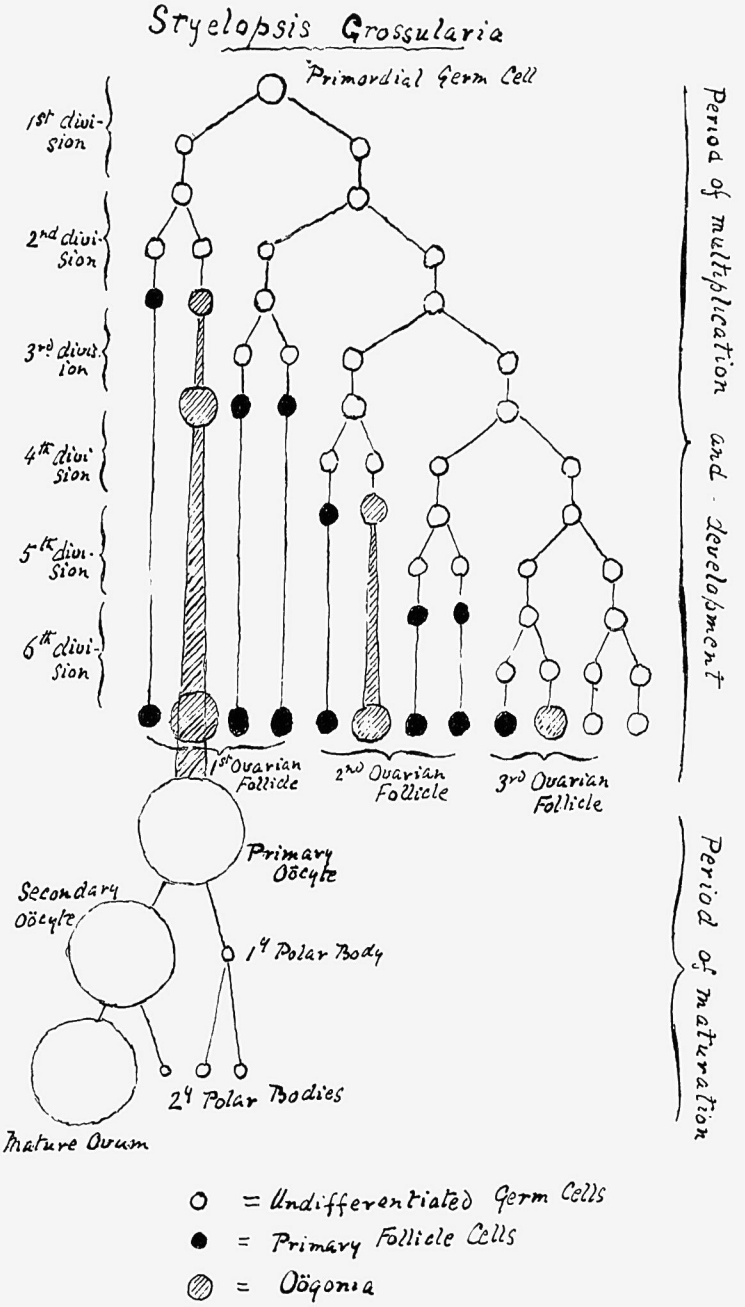
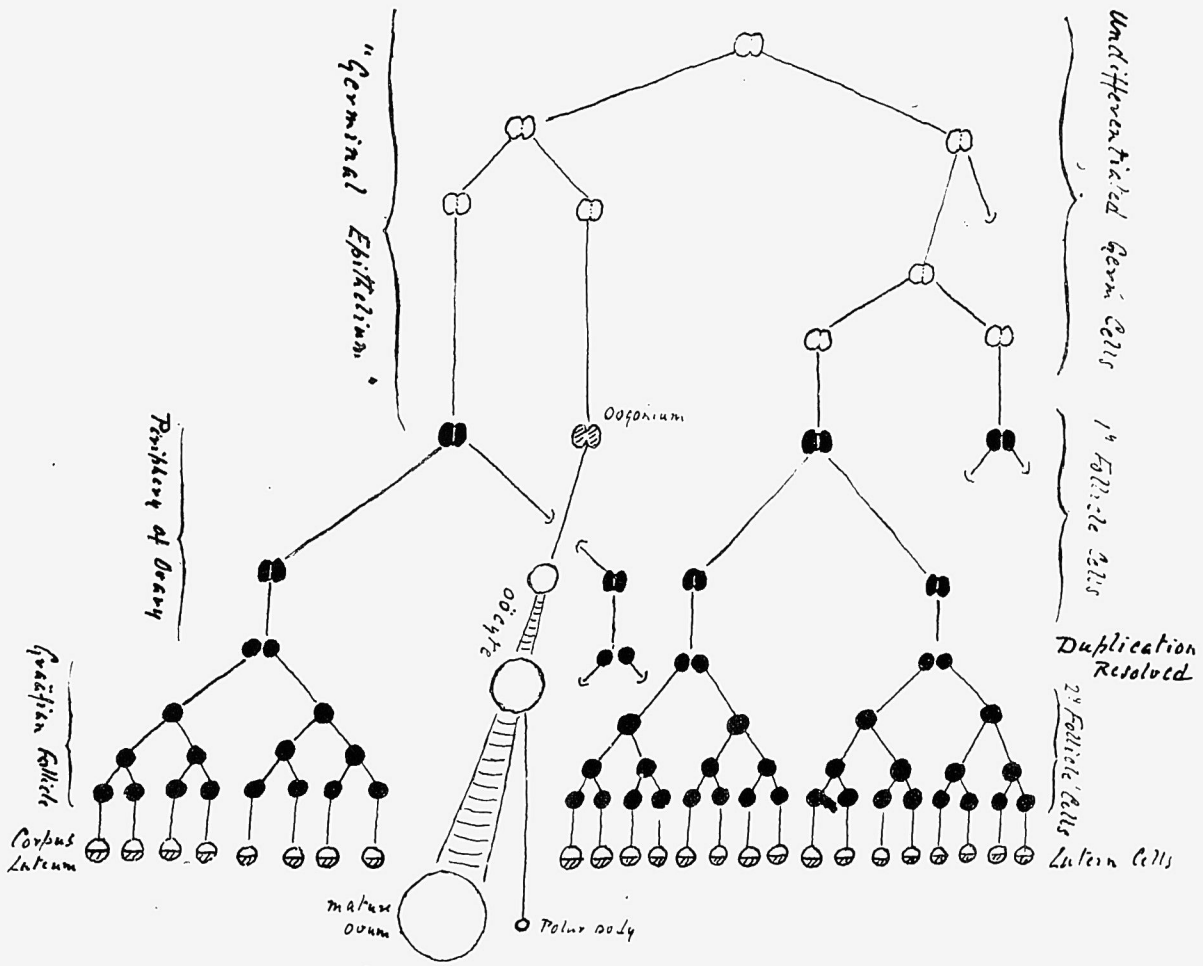

Styelopsis Grossularia
Primordial Germ Cell
1st division
2nd division
3rd division
4th division
5th division
6th division
Period of multiplication and development
1st Ovarian Follicle
2nd Ovarian Follicle
3rd Ovarian Follicle
Primary Oöcyte
Secondary Oöcyte
1st Polar Body
2d Polar Bodies
Mature Ovum
Period of maturation
= Undifferentiated Germ Cells
= Primary Follicle Cells
= Oögonia

Fig. I

Suggested scheme to illustrate the
Life History of the Follicle Cells in the Mammalian Ovary



Only a portion of the divisions are represented.

Fig. II.

of its offspring in succeeding generations of beings, while its less fortunate brethren must find a comparative early grave enshrouded in the mysteries of the corpus luteum. Herein would lie one of the fundamental processes of inherited character, a struggle for the division of chromatin among a series of such struggles which would eventually determine the characteristics of succeeding generations.

In the foregoing remarks the possible relation of follicle cells to reproductive cells has been regarded merely from anatomical and morphological standpoints. The physiological aspect is worthy of passing notice. That the ovary taken as a whole, exercises some obscure functions in exciting and maintaining the sexual characteristics of the individual is beyond question. This function is by many investigators attributed to an internal secretion on the part of the interstitial cells, while the follicle cells have been generally regarded as merely an epithelial covering protecting the ovum during maturation. The ideas that prevail on this subject are based chiefly on observing the results of removal or transplantation of the ovarian tissues *in toto*. There is no reasonable evidence by which we may attribute this function to the interstitial cells with certainty. The character of the follicle cells as described above would rather tend to influence us in supposing that they are not only related anatomically to the ovum, but that being in themselves related to reproductive cells, their function is also connected with those characters which are necessary to induce and facilitate the reproduction of the species. Reference has already been made to the fact that the period of life in both follicle cells and reproductive cells is practically synchronous, in so far as the existence of the latter in the host of one generation is concerned; that is to say, in the individual animal to which these cells belong. The life history of the follicle cells may be divided into four periods, each of which is associated with a synchronous period in the history of the ovum. Thus we have a genetic period, a period of rest, a period of active growth and division, and, lastly, a retrogressive period during which the follicle cells enter into the yellow substance of the yellow bodies. These periods correspond with four periods in the life of the ovum—a genetic period, a period of rest, a period of maturation, and a period of retrogression in utero, or, if fertilised, a period of renewed growth. It is remarkable, that so intimate is sympathy between follicle cells and ovum, that the period of existence in the former as the lutein cells should be influenced, whether directly or indirectly, by the life of the ovum in a comparatively remote organ, the uterus. So great is the sympathy or influence that the event of pregnancy prolongs this period until after the embryo is completed. Again these periods are associated each with definite and peculiar phases in the life of the individual—the period of embryonic life, the period of childhood, the period of sexual activity, and lastly that of senility when the follicles have ceased to mature. In a word, the life of the ova is intimately

associated with the life of the individual throughout all periods, and the life of the follicle cells is associated with that of the ova, and their most active period of growth is the one in which the sexual activity of the individual is prevalent.

Reverting to transplantation experiments, the observations of Limon,[14] Knauer,[15] Grigorieff,[16] Ribbert,[17] and many others have established the power of regeneration on the part of all the essential tissues of the ovary with the exception of the "germinal epithelium," and have shewn that the sexual characteristics largely disappear and later reappear with the degeneration and the regeneration of these tissues respectively. Similar experiments (not published), have been carried out by the writer. It would appear that the periphery of the organ is the last to degenerate and the first to regenerate, and that the follicle cells never undergo true degeneration, nor do they form true yellow bodies. The change is rather retrogressive than degenerative. When the follicles regenerate they undergo changes similar to those which occur at puberty and their development and growth takes place at the earliest possible period, namely when the interstitium has sufficiently recovered to permit of the follicles obtaining their nutrition; for the follicles, unlike the interstitium, are non-vascular. The restitution of the sexual characteristics is generally tested by putting the female animal, whose ovaries have been transplanted, to the male, yet there was no evidence to shew that she would receive him before such a period of time had elapsed as to permit of the complete rejuvenescence and growth of the follicle. In the case of the rabbit this period is about three-and-a-half months. While this result corresponds with that of the writers referred to, it is remarkable that none of them give due prominence to the follicle cells in forming their conclusions. So far, then, as these experiments have gone, they are not sufficient to shew that the sexual characteristics depend only, or depend at all, upon the interstitial cells, while the evidence is rather in favour of this phenomenon depending in part, at any rate, upon the follicles or the ova which they contain. The experiments *are*, however, sufficient to shew that the follicle cells are endowed with a high power of resistance when under the influence of starvation, while their capacity of regenerating at the earliest possible period points to a natural vitality of a high degree. In view of what follows it has yet to be determined whether this vitality depends upon an intrinsic and active property of the cell itself or whether it is maintained by reaction or interaction with other influences from outside, as, for example, with the internal secretions of other glands, such as the pancreas or thyroid.

Pending further developments the recent investigations of Fraenkel and others into the functions of the *corpora lutea* are passed over, though their significance has been fully realised.*

* The subject has been summed up in a recent article by H. R. Andrews (see references—18).

THE SUPPOSED RELATION BETWEEN THE SEXUAL GLANDS AND MALIGNANT NEOPLASMS.

With reference to the problems of cancer and other malignant neoplasms, it is intended to state a few obvious facts that would lead a student of cancer to study the nature of the Graäfian follicle, and to offer some suggestions as to the possible relation of follicle cells to cancer cells, in so far as they may touch upon some already current hypotheses. Any addition to the multifarious theories and hypotheses that already exist, will be avoided as far as possible.

A connection between the metabolism of a cancer and that of the reproductive glands had been suspected long before Farmer, Moore and Walker,[6] in their now historical paper, to which reference has already been made, shewed that there was actually a subtle morphological resemblance between certain cancer cells and the gametogenic cells of these glands.

The removal of ovaries from animals suffering from cancer is generally followed by a certain amount of shrinking in the cancer tumour. In women this operation has been known, on more than one occasion, to have resulted in a practically complete disappearance of the tumour, where such has been situated in the mamma. The valuable work of Beatson[1] in this connection is well known, and was largely responsible for stimulating the investigations described in this thesis.

Ovarian extracts have been frequently administered empirically in many hospitals to patients suffering from cancer. The unsatisfactory results of such administrations are not surprising, when one reflects that, if only in view of what occurs in follicle cells, there must be more than one bio-chemical influence at work among the reproductive glands. Further, dead extracts could only be expected to be of therapeutic value on the supposition that they are of the nature of true ferments. To say that all the bio-chemical substances connected with the ovary are of this nature would indeed be a sweeping statement. Even if this were the case there is reason to believe that there is more than one active product connected with the ovary, and it is impossible to tell what may be the combined effects of the supposed ferments which may be connected with the maturing follicle, the corpus luteum, and the interstitium respectively, upon each other and upon the body generally. Lastly, the undoubted influence of other internal secreting glands upon the sexual glands must not be forgotten.

The recent work of Beard[3] on the distribution of germ cells outside the reproductive glands as evidenced in embryos of *Raja batis* and other fishes, renders the theory as to the germ cell character of malignant tumours,

to say the least, possible, and establishes the possibility of a relation between cancer tissues and reproductive tissues that is not only structural but also embryological or parental.

Lastly, it has been long recognised that the period of life in which the great function of the reproductive glands is at its zenith is the one period in which malignant tumours are the least prevalent.

Bearing these facts in mind, and remembering the character of malignant tumours in general, it is significant that in the Graäfian follicles we should have examples of natural tumours actively growing in a tissue of another character, namely the interstitium. It is noteworthy that these natural tumours, growing most rapidly at the reproductive period of life, are most in evidence at that period in which malignant disease is least in evidence. In view of what we know to occur in certain commencing cancers, more especially in epitheliomata, and bearing in mind their supposed origin from a germ cell, it is worthy of consideration that the Graäfian follicles should be derived from cells that have sprung from an ingrowth of the integumentary layer of the organ in which they lie, and that after a long period of rest they should assume an active growth at a definite period in the life history of the individual of whom they form a part.

These facts were considered sufficient to warrant the devotion of much time to the object of studying the minute structure of the follicle cells in the mammal, and it is certainly encouraging to find that there are in the Graäfian follicles more minute and more substantial resemblances to the malignant tumours than the foregoing remarks would imply; for in these follicle cells it would almost seem as if we had found cells, which, in their minute structure and in their method of growth and division, as well as in their early history, bear a striking resemblance to the cells of malignant tumours. Their nature as revealed in their occasional heteromitotic character may be calculated to throw a brighter light on the significance of heteromitosis in malignant structures than has hitherto obtained in the true gametogenic cells, or cells whose development results always and exclusively in the formation of the terminal sexual unit.

For although cancer cells may resemble reproductive or sexual cells by virtue of their heterotype character, we have hitherto been faced by the fact that sexual cells cease to multiply almost as soon as they have assumed the heterotype process of dividing. In mammals only one such division occurs ere the cell is at rest, awaiting its lost complement of chromosomes from a similar cell of the opposite sex, whereas, in the case of the cells in malignant tumours, heteromitosis is associated directly or indirectly with unlimited and indefinite growth. Cancer would thus seem to contradict nature and consequently we have been met by a serious problem that has not yet been explained, though an ingenious attempt has been made to do so by Bashford,[19] who has endeavoured to establish the phenomenon of "conjugation" in these tumours.

The possibility of conjugation, in the true sense of the word, occurring between a germ cell and a somatic cell, is, however, very doubtful, when regarded from an embryological standpoint, though, could such a phenomenon be established, it would explain much in connection with cancer.

The presence of heteromitosis occurring among follicle cells seems at once to throw a new light on cancer, while it darkens our conception of the true meaning of reduced chromosomes, and renders it necessary to revise our views as regards the significance of the polar bodies, and of the essential morphological character of the sexual cell in both sexes; for reduction can no longer be regarded *per se* as the essential factor in the formation of a sexual unit. It throws light on malignant growth, inasmuch as we need no longer regard the heteromitoses, which separate these growths from the benign ones, as being in themselves of pathological significance, for it would seem that such mitoses may occur in actively growing tissues of nature, provided such tissues are of a germinal character. The observations would further seem to render the hypothesis of conjugation unnecessary. It is possible that the initiation of malignant growth is brought about by influences, perverted or pathological, acting upon germ cells in such a way as to resolve their duplicated nuclei into their parent halves and to thus bring about a malignant tumour manifesting the heterotype division. The writer would venture to suggest that much of what has been regarded as a manifestation of conjugation among cancer cells is, in many cases, nothing more nor less than a process of duplication such as obtains in the early follicle cells and in germ cells generally previous to the reduction of chromosomes and previous to the heteromitotic process.

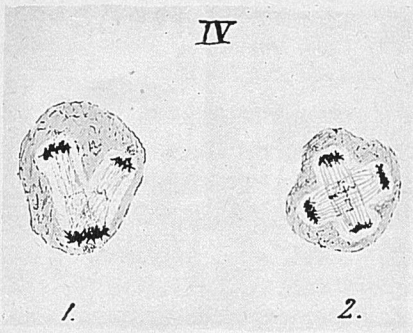
To draw a closer homology between what we may call "a natural follicle tumour" and a malignant neoplasm may appear rash and far-fetched, for the differences as revealed in the history, structure, and behaviour of the two growths are as striking as the resemblances; yet may we not explain much if we remember that one is natural and the other abnormal, if not pathological? Also that the one is destroyed while still very young under the influences of extra-ordinary changes in the *corpora lutea*, while the other is initiated and maintained by some influence or by some etiological factor as yet undiscovered. We have seen that the recent investigations of Farmer, Moore and Walker suggest that cancer genesis and gametogenesis are indirectly allied to one another, and it has been suggested that a malignant tumour is possibly the result of misconducted germ cell activity. This theory, which has been persistently held by Beard and recognised, if not accepted, by A. S. Grünbaum,[20] is too tempting a one to refuse with our present knowledge.* Yet, if we accept it, we have still to discover of what nature that influence or those influences are which stimulate

* The germ cell origin of cancer is, in this paper, regarded in a general sense only. The source from which such cells are derived, whether from the trophoblast or otherwise, is not discussed.

germ cells into activity at a comparatively late period in the life of their hosts. In the case of reproductive cells, to which we must add follicle cells, this influence must be natural.* In the case of cancer cells it must either be a natural influence that has become perverted, or else it must be truly pathological. The mitotic divisions, which occur in cells under the influence of pathological reaction, are generally unequal; that is to say, that the chromosomes are not only unequally divided but are also unequally distributed to either pole. Such divisions have been artificially produced by Galeotti[21] in the epithelial cells of the Salamander by the exhibition of various drugs, and the fact that they are so common in malignant tumours certainly suggests that the influence which initiates the growth of these tumour cells, as such, is a pathological one. The phenomenon is certainly not connected with heteromitosis, as it is not to be found in the follicle cells nor in other normal heteromitotic growth, either in plants or animals. Multipolar division, which is generally associated with pathological and rapid growth, may, however, occur in normal tissues, and the writer has found both a triaster and a tetraster among the cells of an apparently healthy ovarian follicle (see Plate IV.). It is interesting to note that Strassburger and Mottier[22] have described these multipolar figures in the pollen mother cells and endosperm cells of flowering plants, though in this case they are said to arise through the mingling of one amphiaster with another. They are not uncommon in germ cells and in Beard's paper on the germ cell they are described as being prevalent in the "Megaspheres" of the embryo, and are regarded as being connected with retrogressive changes. They also have been artificially produced by Schottländer.[23] Multipolar division, then, by virtue of its occasional occurrence in the Graäafian follicle, and in view of Strassburger's observation, may be claimed as one more factor suggestive of the germ cell origin of the follicle cells, while in cancer it may be regarded in the same light, or like asymmetrical mitosis, as a manifestation of a pathological process. Possibly its comparatively frequent occurrence in these tumours is the result of the action of a pathological influence upon germ cells.

It is the fashion nowadays, in this country at all events, to discountenance those theories which seek to explain the growth of cancer as a manifestation of organismal infection, while those who accept the germ cell theory as to the nature of these tumours, are apt to regard it as being not only indicative of their origin but also as an explanation of their initiation, maintenance and growth, which they regard as being brought about by an influence that is exclusively intrinsic to the cells themselves. If the homology between follicle growth and malignant growth can be accepted, is it not reasonable to explain the differences that exist in these two structures as being partly

* The word "natural" is here intended to qualify those influences which occur as a functional process in the metabolism of tissue life, as opposed to pathological. The word "physiological" might have been employed, but this also is open to objection.



Follicle Cells shewing Multipolar Division.

1. TRIASTER. 2. TETRASTER.

These figures were discovered in the same section of the same follicle. They were the only multipolar figures found throughout the whole investigation. The type of mitosis is in both cases somatic.

dependent upon outside influences and not exclusively upon any intrinsic difference between the cells themselves? It is possible that a germ cell is by heredity endowed with a natural power of marking time, as it were, after which, of its own initiation and independently of outside influences, other than those connected with its nourishment, it seems to spring into active growth. However incomprehensible this may seem to be, the observations of embryologists are such as to lead to the belief that this is the actual process of nature. It would be a sweeping statement, however, to say that germ cells, however independent it may be possible for them to be of outside influences, are in all cases independent of such influences, or that their activity is incapable of being arrested or invigorated by substances derived from the host in which they grow. It is known that germ cells of the lower organisms, such as the protozoa, are capable of being stimulated to division by chemical products, by new environment, by alterations in their nourishment, and by changes of temperature. The natural tendency to assume active growth at the time of puberty on the part of the Graäfian follicle finds a parallel in somatic tissue amongst certain of the hair follicles, also in the vocal cords of mammals and birds, also in the plumage of the latter, and in the integuments of many fishes,* which at puberty are stimulated into proliferation, apparently by internal secretions connected with the glands in which the germ cells normally reside. Indeed the normal position in which germ cells are found in extra-uterine life, namely, in the reproductive glands, and the influence that these glands undoubtedly have upon those characters which are connected with the reproductive functions of the individual, suggest that the forces or substances which normally react upon the normal germ cell throughout the different stages of its history, maintaining it at rest, or stimulating it to growth, are derived from products either elaborated by the reproductive glands themselves or, as is more probable, by the interaction of other products of the body with those of these glands.

It has been suggested above, that in the case of the ovary, the follicles themselves may play an important part in maintaining the sexual characteristics of the individual. It may be further suggested that they may be concerned in the maintenance of their own growth by virtue of a reaction or an interaction with essential products derived from elsewhere, that is to say, products over and above those which are generally required for the growth and upkeep of the tissues in general; but whatever their influence may be, and by whatever factors they are themselves influenced, it is probable, taking into consideration their history at the different periods of animal life, that there is some factor acting from outside without which they would be incapable of suddenly springing into active growth, or else without which they would be incapable of maintaining

* There are many other examples, see Darwin's "Origin of Species"—The Evolution of Sex.

a long period of rest. In the case of the follicle cells and the gametogenic cells of both sexes this factor must be a natural one, but in the case of cancer it may be an unnatural one, and on this may depend the irregularities of division of cancer cells and many of the other characters by which they differ from follicle cells. Whatever may be the extrinsic influence connected with the growth of follicle cells, it is counteracted so soon as the ovum and liquor folliculi are expelled from the follicle, whereas, in the case of cancer it is not so counteracted, and would appear to become, to some extent, accumulative.

If, on the other hand, and there is much in favour of this theory, germ cells are to be regarded as being, under favourable circumstances, endowed with the powers of unlimited growth, then, the factor to be sought for is that which is capable of counteracting this power and maintaining the germ cell at rest. In the case of normal germ cells this factor must be intimately connected with the reproductive glands, and in the case of the ovary *with the individual follicles*, for while one follicle is at rest another is growing. In the case of cancer it must be largely derived from a different source, for when once counteracted it is apparently incapable of re-asserting itself, that is to say, that the cancer grows until it is killed by the death of its host, and while asserting itself in other parts of the body, the growth is continued both in the primary and in the secondary tumours at the same time.

If during the growth of a cancer, no matter in what part of the body it may primarily take root, there were not elaborated some active extrinsic agent capable of either stimulating the growth and division of cancer cells, or of counteracting any supposed influences that may keep such cells at rest, it would be difficult to explain why any organ, such as the ovary, in which a primary cancer is comparatively rare, should be so resistant to primary cancer but not so resistant to secondary growth when once cancer has asserted its influence over the individual in whom it grows. There must be some general active agent interacting with the primary growth and capable of lowering what was formerly a high power of resistance to one that is capable of permitting the metatstatic tumour to proliferate.

It is possible that an organ capable under normal circumstances of resisting the overgrowth of germ cells situated within it, and which has been for a long time held at rest by the metabolism that takes place within that organ, is not so resistant to the inroads of cells derived from another organ, when once the germ cells have been successful in overcoming the resistance of this latter organ and in forming within it a primary cancer. In other words, the power of resistance to the growth of the original cancer cell or germ cell may be acquired by virtue of some interaction that has become familiar between the germ cell or cancer cell and the organ in which it lies, but in the event of the descendants of this cancer cell finding a nidus within another organ which has been

unaccustomed to the presence of such cells, this organ is incapable of resisting the inroads of these cells, over which it has not had the advantage, so to speak, of a prolonged acquaintance. There having been no period of interaction between this latter organ and the foreign cells derived from elsewhere, this latter organ is incapable of either resisting the growth of the foreign cells or of modifying their structure to its own model.

But this, again, would imply the presence of some influence extrinsic to the original cancer cell, which in this case would be connected with the organ in which such a cell has been situated, and which is capable of maintaining the cancer cell at rest. It further implies that the extrinsic influence is largely a property of the organ in which the cancer grows, and that its power and its nature differ in the different organs of the body, or it may be that these organs react differently to the so-called internal secretions.

It is not suggested that the structural differences that are so apparent between follicle cells and cancer cells, and also between the various forms of cancer cells themselves, are of necessity entirely independent of different intrinsic properties, but it is suggested that there is over and above such intrinsic properties other extrinsic agents at work which must determine partially, if not entirely, the difference in the character of these cells as regards their growth in relation to the organs in which they come to be situated.

It is not, however, intended to enter into this complex subject in detail, involving as it does the whole problem of tissue metabolism, while the structure of the cells of malignant tumours in so far as they differ from one another, is outside the subject of this paper, excepting in so far as the subject may help to explain the difference in structure which would seem to separate the follicle cells from their supposed malignant cousins.

It is convenient here to insert a short note on cancer of the ovary itself. The structural character of follicle cells is very similar to what obtains in a small round cell sarcoma, and this resemblance is alone sufficient to justify the possibility of the connection between the two growths being one which, in view of what has been stated above, may not be remote. Cancer cells differ structurally from one another as much and sometimes more than they do from follicle cells. The rare primary cancer of the ovary (the writer has examined three such tumours in a young stage) resembles nothing more nor less than an accumulation of follicle cells exhibiting irregular mitoses, which, being unchecked in their growth, have infiltrated the surrounding interstitium, and have, in the central and older part of the tumour, united to form an irregular mass. The secondary cancers of the ovary are of a different nature inasmuch as they are derived from cells nodified by other organs. A malignant ovarian cyst is also of a different character, and from whence it is derived is an interesting mystery. The

comparatively uncommon but well-known primary cancer of the ovary, however, is apparently derived from the follicle cells themselves, and would seem to form, therefore, an example of a germ cell tumour derived, not from some misplaced germ cell which has undergone modification in an abnormal position, but from germ cells of the true primitive type, which have already by nature assumed the heterotype characters similar to those which may be found in infiltrating tumours, and which are situated in the organ that forms their natural habitation. Primary cancer of the ovary, therefore, may form a model or fixed point from which to study and compare the phenomena of other malignant growths. Inasmuch as follicle cells resemble sarcomatous round cells, primary cancer of the ovary is frequently mistaken for sarcoma, while the normal follicle cells and the round cells of sarcoma may, perhaps, be both regarded as being derived from germ cells of a primitive or unmodified type, or, in accordance with what has already been put forward from cells on which the surrounding tissue, having for some reason failed to react, have retained their original character up to and after the period in which they spring into active growth.

With reference to the active power of infiltration so characteristic of malignant tumours, and the tendency to metastatic growth, which would appear to result from infiltration of the tumour cells into the blood or lymph stream, we must, of course, admit that we are herein faced by an outstanding feature which at once distinguishes sharply between the growth of malignant tumours and that of the normal ovarian follicle.

It must be admitted, however, that as compared with malignant tumours the ovarian follicle is only, so to speak, at the very early stage of a primary growth when its progress is abruptly arrested at the moment at which the ovum and liquor folliculi are expelled. Furthermore, may not much be explained by the fact that the follicle is a normal growth holding a natural position in a healthy tissue, specially prepared for its reception, and in which the balance of action and reaction, activity and resistance, are kept up by natural influences? Until we can explain infiltration, it is too soon to regard it as a proof of any essential difference between the nature of follicle growth and malignant growth; and whereas the resemblances between these two growths are so striking, we may hope that by seeking for that which prevents infiltration in the one we may be led nearer to finding that which excites it in the other.

Before leaving the question of infiltration it would be well to take passing notice of a subject which may possibly be connected with this problem. The planes or directions in which cancer cells divide bear a relation to one another that is extremely irregular. That is to say that the axis uniting the pole of any one cell is in a direction that bears no definite or constant relation to a similar axis in any neighbouring cell. This must be a constant feature of any growth

of indefinite shape. In a cancer, the irregularity of the planes must be largely dependent upon the fact that the chromatin is unequally distributed to either pole of the nucleus, as a consequence of which one daughter nucleus may be considerably larger than the other; the directions of division and the activity displayed by each of these nuclei must, therefore differ from one another. Irregularity of the chromatin elements must, therefore, untimately result in irregularity in the shape and consistence of the whole tumour.

Now, in order that a spherical body may be produced by the growth and division of spherical cells, the planes of division would be so arranged as to be both concentric with the circumference of the sphere to be produced and also at right angles to the circumference (*i.e.*, radial). Furthermore, the radial splits would be more frequent than the concentric splits, and the increasing frequency of radial splits to concentric splits would accelerate as the sphere advanced in size.*

The follicles, when at a young stage of their existence, are, especially in the rabbit, approximately spherical, and the planes of division adopted by the follicle cells tend to adhere to these mathematical rules. As the follicle grows, however, the development always tends to be more advanced in one part than in any other part, the position of the yet unformed *discus proligerus* can be thus foretold, and the ovum is pushed away from the centre. The planes of division, however, still have a tendency to obey the mathematical model. When the liquor folliculi develops the nuclei are thrown into irregular positions, a strain is put upon the follicle cells, the daughter nuclei of a dividing cell may be seen to shunt from one another (plate I., fig. 9), and the planes of the mitotic figures make no attempt at any form or degree of regularity. The investing tissues of the follicle are bent by the strain, but any further history is left to the imagination for the follicle now bursts, the egg and liquor folliculi are expelled, and the follicle cells are once more at rest.

While the planes of division are largely influenced by pressure exerted from the surrounding tissues, there seems to be some property belonging to the dividing cell which largely determines these phenomena. The influence of the surrounding tissues, however, can be easily realised when one compares the shape, for example, of a cancer nodule in the liver with that of an epithelioma of the skin.

The comparison of follicle growth with malignant growth might be pursued further, but for the present it is unnecessary. It is sufficient to note that under the assumption that malignant tumours are of a germ cell origin they bear a certain relation to follicle growth, that with the growth of germ cells there is probably connected some extrinsic influence derived from their host, which is capable of reacting upon such cells, and that this influence, whatever it may be,

* I am indebted to Major Ross for working out for me this mathematical problem, of which I give the solution only.

is probably of a somewhat different nature in each case; that in the case of follicle growth it is natural, and that in the case of cancer it is either a natural process perverted, or it is of the nature of a truly pathological poison reacting upon such processes. We have seen that the irregular nature of the cancer cells is such as to favour the latter hypothesis in these tumours, but remembering the effect that may be produced upon a cancer by removing the ovaries of the affected animal, we are bound to admit that any pathological process that may be involved is probably one which is capable to some extent of interaction with the internal secretion of the sexual glands. We also note that while there is some connection between the influences that bear upon the proliferation of follicle cells and those of malignant neoplasms, there is also a striking resemblance between the two forms of growth which is borne out by details of their origin, their derivation, their history, their relation to the tissues in which they grow, their structure, and, most important of all, by the minute details connected with the process by which they undergo division and proliferate.

It must be remembered that the pathology of the tissues has been hitherto studied almost exclusively in so far as it influences somatic cells. The pathology of germinal tissue is almost an unknown subject. What scant attention has been paid to the effects of simple orchitis, for instance, upon the growth or atrophy of the spermatogonia, and yet how frequently does sarcoma of this organ follow this disease? How much may there not be learnt by watching the effects of organisms upon a simple adenoma, and how little do we know of the influences of toxins, natural or pathological, upon the mitotic divisions of germ cells! The Graäfian follicles may form a convenient field of research in determining the influence upon germ cells of any organisms which may from time to time be isolated from malignant tumours, or which may be credited with a power of exciting germ cells into activity. It will, however, be necessary to bear in mind that in carrying out such experiments upon the Graäfian follicle, we are dealing with germ cells which by some remarkable and obscure phenomenon are arrested in their growth while the tumour is still minute, and that, after being thus arrested, some of them undergo a most remarkable and unparalleled metamorphosis into what is known as the yellow substance of the corpus luteum, where, after lying idle for a limited time, they are subsequently reduced to a simple scar.

When first led by the observations of others to seriously regard the germ cell origin of cancer as something more than a fanciful theory, hopes were dimmed in proportion as interest was excited. The immense vitality of germ cells, their persistence, their power of preserving their individuality, their inherent parasitic qualities, revealed in cancer, if from such an origin it be sprung, a growth that no conceivable power could stay without destroying the life of the

individual in which it takes root, a growth, it seemed, which must live or die with the tissues which itself destroys. Yet if we regard the life of the follicle cells, their great activity and their subsequent destruction, are not our hopes once more raised, if in them we have identified germ cells exhibiting that very type of proliferation by which we characterise the malignant neoplasms, and yet a type of proliferation which can by nature be brought to an abrupt ending in those cyclic changes which are associated with ovulation, and which destroy their own artifice more rapidly than they build it up. Herein lies a hope that we may ere long discover a means by which we may so alter the balance of tissue metabolism, or the various internal secretions connected with it, as to induce nature to eliminate and destroy cancer or tumours of germ cell character, and yet to preserve the life of the body as a whole.